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Russula brunneovinacea sp. nov., from northeastern China

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Abstract—*Russula brunneovinacea* is described as a new species from northeastern China. It is characterized by its deep brownish vinaceous to Etruscan red or Kaiser brown pileus with a slightly depressed center, incurved margin with striate, Naples Yellow context that dries with a greyish tinge, subglobose to broadly ellipsoid basidiospores with an amyloid plage and isolated warts that are connected at base or in ridges warts but do not form a reticulum. The new species is supported by ITS phylogenetic analyses and tentatively placed in *Russula* subsect. *Integroidinae* based on morphological analyses.

Key words—macrofungi, *Russulaceae,* taxonomy

Introduction

Russula Pers. (*Russulaceae*, *Russulales*, *Basidiomycota*) was erected by Persoon (1796) and with a worldwide distribution (Kirk et al. 2008). The genus, which is ectomycorrhizal, includes many important edibles such as *R. griseocarnosa,* popular in China and Southeast Asia (Wang et al. 2009, Li et al. 2010, Zhao et al. 2015) as well as poisonous species such as *R. emetica* (Schaeff.) Pers. and *R. subnigricans* Hongo (Miller & Buyck 2002, Yang & Piepenbring 2004, Li et al. 2015).

Russula subsect. *Integroidinae* (Romagn.) Bon was validated by Bon (1986), based on *R.* sect. *Integroidinae* Romagn. (Romagnesi 1958). Subsequently Bon placed the subsection within *R.* sect. *Lilaceae* (Bon 1988). In this paper, we follow the taxonomy of Bon (1986), who characterized species within this subsection by a pileus with red, yellow, purple and complex color, a grayish

context with a rarely blackening tinge, pileocystidia that are absent or rarely septate when present, and sometimes with primordial hyphae.

During our research on species diversity of *Russula* in the Changbai Mountains of northeastern China, we identified two interesting *Russula* collections as a novel taxon, which we tentatively place in *R.* subsect. *Integroidinae* based on detailed morphological examination.

Materials & methods

Specimens, collected from Changbai Mountains, Jilin Province, during 2012, were photographed in the field, with macroscopic descriptions of the fresh fruiting bodies based on the photos and notes made in the field. After being dried at 50–55°C with an electric drier, the collections were deposited at the Research Institute of Tropical Forestry, Chinese Academy of Forestry Sciences, Guangzhou, China (RITF). Terminology for descriptive terms follows Vellinga (1988). Color names and codes refer to Ridgway (1912).

For microscopic observations, sections of specimens were cut by hand, mounted in 5% KOH, and then stained with Congo red. We used Melzer's reagent to determine spore amyloidy and Cresyl Blue to test spores for a metachromatic reaction (Singer 1986). Sulfovanillin (SV) was used to test for reactions of cystidia. Sizes of basidia, basidiospores, cheilocystidia, pleurocystidia and elements of the pileus covering were measured using the MShot Digital Imaging System. The abbreviation [n/m/p] denotes measurements made on 'n' basidiospores in 'm' basidiomata from 'p' collections. Basidiospore dimensions follow the notation (a–)b–c(–d); the range 'b–c' includes 90% of the measured values with the extreme values ('a', 'd') given in parentheses. The spore quotient Q indicates the length/width ratio of spores, and Qav denotes the average Q of all basidiospores ± sample standard deviation.

DNA was extracted from dried materials with an improved CTAB protocol (Zhou & Liang 2011). Sequences of ITS region were amplified with the primers ITS5 and ITS4 (White et al. 1990). PCR products were purified using the Bioteke DNA Purification Kit. Both strands were sequenced with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology & Services). These newly generated sequences were submitted to GenBank.

DNA sequences were edited and aligned by SeqMan (DNA STAR Package) and MUSCLE (Edgar 2004) and manually checked and adjusted. Ambiguously aligned positions were excluded from the matrix. Gaps in alignment were treated as missing data. All unambiguous characters and character transformations were equally weighted. A blast search of the query sequence in GenBank was performed to determine the most closely related taxa for molecular identification. A dataset was generated from the highest scored hits most relevant for identification, as well as from samples previously used in the phylogenetic reconstruction of *Russula* (Miller & Buyck 2002). *Russula virescens* (Schaeff.) Fr. and *R. foetens* Pers. were chosen as outgroup taxa.

Maximum likelihood (ML) and Bayesian analyses were used to generate a phylogeny. The ML tree generation and bootstrap analyses were performed with the RAxML BlackBox online server (Stamatakis et al. 2008). All parameters in RAxML analysis were at default, statistical support values were obtained using nonparametric bootstrapping with 1000 replicates, and trees obtained prior to convergence were discarded before the consensus tree. A bootstrap proportion greater than 75% is considered significant. The results were subsequently exported to Dendroscope for tree viewing and editing (Huson et al. 2007).

The dataset was further analyzed with MrBayes 3.1 (Huelsenbeck & Ronquist 2005) under a general-time reversible (GTR) model (nst = 6) and the parameter model was selected by the Akaike Information Criterion (AIC) as the best-fit likelihood model with Modeltest 3.7 (Posada & Buckley 2004). Bayesian analyses were run 6 chains for 1.0 million generations with sampling every 1000 generations. Trees were pooled together and used to generate a 50% majority-rule consensus tree with branch lengths. Nodes that were recovered more than 95% of the time were considered to have a significant posterior probability.

Taxonomy

Russula brunneovinacea X.M. Jiang, Yang K. Li & J.F. Liang, & **sp. nov.** Fig.1

MycoBank MB 819154

Differs from *Russula griseocarnosa* by its deep brownish vinaceous to etruscan red or kaiser brown pileus, barium yellow spore print, and smaller basidiospores with smaller spines.

Type—China, Jilin Province, Changbai Mountains, Lushuihe hunting ground, alt. 1000 m, 21 August 2012, Zhang Xin 268 (**Holotype**, RITF 2242; GenBank KY114148).

Etymology—The Latin word "*brunneovinacea*" refers to the brownish vinaceous color of the pileus.

Basidiomata medium. Pileus 5–6 cm in diameter, hemispherical when young, expanding to applanate or concave with a slightly depressed center, surface viscid when young and moist, glabrous when mature; Light Coral Red (xiii5′b) to Coral Red (xiii5′) when young, Dark Vinaceous (xxvii1″) to Mineral Red (xxvii1″k) or Burnt Sienna (ii9k) at centre after maturity, margin slightly incurved, sometimes undulate and dehiscent, obviously striate, Deep Brownish Vinaceous (xxxix5‴) to Etruscan Red (xxvii5″) or Kaiser Brown (xiv9′k), often becoming Hay's Russet (xiv7′k) to Russet (xv13′k) or Ocher Red (xxvii5″i) to Prussian Red (xxvii5″k) when dried. Lamellae White (liii) to Cream Color (xvi19′f), adnate, not forked, lamellulae absent, becoming Chamois (xxx19″b) or Salmon Color (xiv9′d) with greyish tinge when dried. Stipe 4.5–5 × 1–1.5 cm, central, cylindrical to subcylindrical, enlarged towards the base, white, often tinged Hermosa Pink (i1f) tinge when mature, Baryta

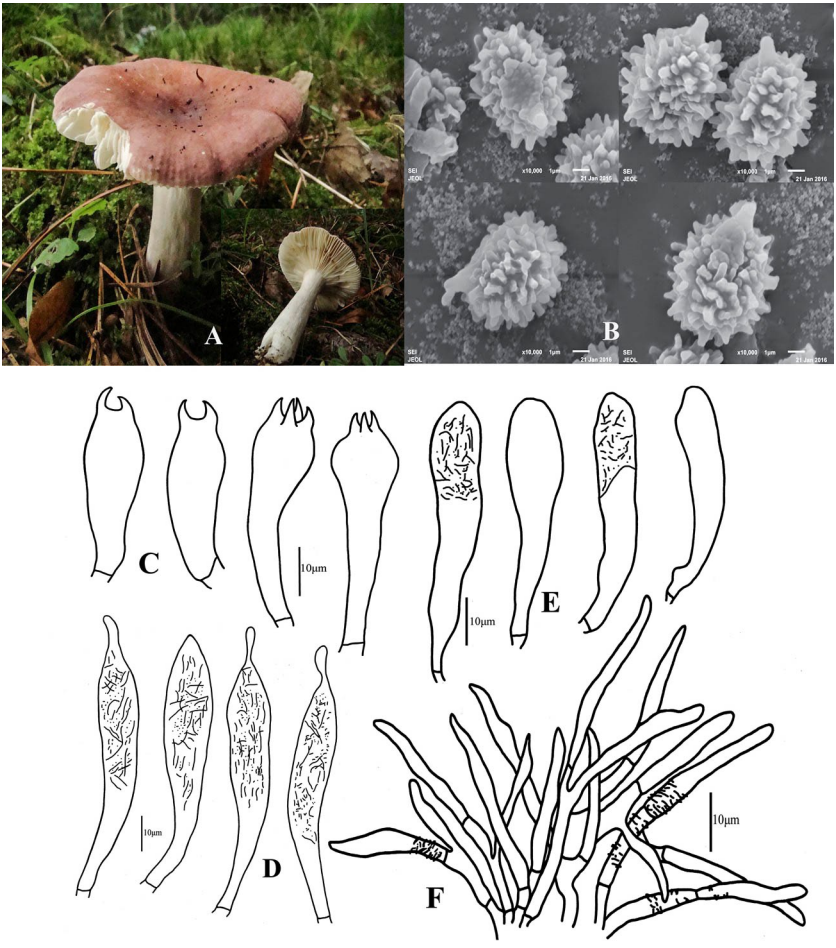

Fig. 1. *Russula brunneovinacea* (holotype, RITF2242). A. Basidiomata; B. Basidiospores; C. Basidia; D. Pleurocystidia; E. Cheilocystidia; F. Pileipellis

Yellow (iv21f) and tinged with Eosine Pink (i1d) when dried, originally solid, then stuffed with a bread-textured trama to irregular cavities, color unchanging when injured. Context white (liii), Naples Yellow (xvi19′d) with greyish tinge when dried. Odor a little sweet. Taste few acrid. Spore Print Barium Yellow (xvi23′d).

Basidiospores [52/2/2] (6.1–)6.2–8.5(–8.8) × (5.1–)5.3–7.1(–7.8) μm, Q = (1.0–)1.03–1.31(–1.43) (Qav = 1.18 ± 0.09), subglobose to broadly ellipsoid,

rarely globose or ellipsoid; ornamentation amyloid; warts bluntly conical to subcylindrical, 0.7–0.9 µm tall, isolated or connected at base or ridges, not forming a reticulum; plage distinctly amyloid; hyaline in 5% KOH. Basidia 32–46(–52) × 10–13.5 µm, narrowly clavate to clavate, inflated towards upper half, 4-spored, rarely 2-spored, hyaline in KOH, sterigmata about 2.5–4.5 µm long. Lamellar trama mainly composed of nested sphaerocytes (28–41 × 26–33 µm) surrounded by connective hyphae. Pleurocystidia 54–89 × 9–13 µm, abundant, narrowly clavate to clavate, apex often with papillate appendage, with abundant granular contents in the upper part, red to slightly purplish red and weakly grey in SV. Cheilocystidia 43–82 × 7–13 µm, clavate with rounded or indistinctly mucronate apex, few with granular contents. Pileipellis composed of hyaline hyphae (2–5 µm diam.), often branched and interwoven, septate; terminal cells 16–37 × 2.2–4.1 µm, cylindrical, with obtuse apex, sometimes attenuate, and some with zebroid encrustations. Clamp connections absent.

Habitat & distribution — Solitary or dispersed in conifer and conifer–broadleaf forests. So far found only from the type locality.

Additional specimen examined — **CHINA**: **Jilin Province**, Changbai Mountains, alt. 1000 m, 19 August 2012, Zhang Xin 226 (RITF 2206; GenBank KY114147).

Phylogenetic analyses

A dataset of 32 ITS sequences with 666 nucleotide sites (including 30 available sequences of *Russula* from GenBank) was analyzed. Both the RAxML and Bayesian analyses resulted in similar topologies and only the best maximum likelihood tree with bootstrap supports is shown (Fig. 2). Bayesian posterior probability is also displayed with the bootstrap values along the branches.

Bayesian and RAxML phylogenetic analyses clustered two collections together and obviously separate from the related sequences available on GenBank. Phylogenetic analyses also grouped the new species with *R. griseocarnosa* X.H. Wang et al., *R. occidentalis* Singer, *R. vinosa* Lindblad, and other species with weak statistical support.

Discussion

Russula brunneovinacea is well distinguished by its Deep Brownish Vinaceous to Etruscan Red or Kaiser Brown pileus with a slightly depressed center, its incurved striate margin, its white or cream lamellae that become Chamois with a greyish tinge when dried, its Naples Yellow context with greyish tinge when dried, its somewhat acrid taste, and its subglobose to broadly ellipsoid spores with small isolated or connected at base or ridges warts.

Bayesian and RAxML phylogenetic analyses support *R. brunneovinacea* as closely related to the Chinese specimens previously misdetermined as "*R. vinosa*" and subsequently described as a new species, *R. griseocarnosa* (Wang et al. 2009). *Russula griseocarnosa* differs from *R. brunneovinacea* by its dark red sticky pileus, white context turning greyish during maturity, white spore print, stipe tapered at base, larger basidiospores (8–10 × 6.5–8 μm) with longer (≤2.5 μm) spines, and pleurocystidia (100–150 × 13–20 μm). Geographically, *R. griseocarnosa* invariably grows in tropical and subtropical China while *R. brunneovinacea* grows in northeastern China with a temperate monsoon climate.

Russula occidentalis is also close to *R. brunneovinacea*. A BLAST query showed that the two species have a 92% maximum identification with 91% coverage. However, *R. occidentalis* has a variably colored pileus, lamellae with forking edges that bruise grey to black, a white to creamy white stipe that stains dingy pink where handled or damaged and becomes dark grey to brownish-grey or black, and bigger spores (7.9–11.2 × 6.7–8.9 μm), basidia (52–62 × 14–17.7 μm), and pleurocystidia (70–125 × 9–13 μm) (Romagnesi 1985, Roberts 2007). *Russula vinosa* has a 90% maximum identification with *R. brunneovinacea;* it differs in its vinaceous red, dark wine-red or somewhat brownish pileus, lamellae becoming grey to black when cut or bruised, pale ochre spore print, mild taste and distinctive odor (Romagnesi 1967, Bon 1988).

In the field, *R. brunneovinacea* is easily confused with the United States species *R. rubescens* Beardslee (Romagnesi 1985). *Russula rubescens* also has red color on the pileus, but differs by its paler margin fading with age, mild taste, forked lamellae, white stipe becoming cinereous, and often blackening with age or upon drying, and quickly becoming red and then black when wounded, and basidiospores with an indistinctly amyloid plage. *Russula xerampelina* (Schaeff.) Fr. and *R. olivacea* (Schaeff.) Pers. (Romagnesi 1985, Roberts 2007) also resemble *R. brunneovinacea* in appearance. *Russula xerampelina* is distinguished by its ochraceous-olive young pileus, non-striate margin, warm yellow to light orange-yellow lamellae often staining brown where damaged, cream to light yellow stipe, crab or shrimp odor, light orange yellow spore print, and basidiospores with longer (≤1.2 μm long) warts, while *R. olivacea* differs in its bigger (10–20 cm) basidiomata, drab pileus with olive green and purple hues, non-striate margin, egg-yolk yellow lamellae unchanging when damaged, deep ochre-yellow spore print, and basidiospores with incompletely amyloid plage (Romagnesi 1985, Roberts 2007).

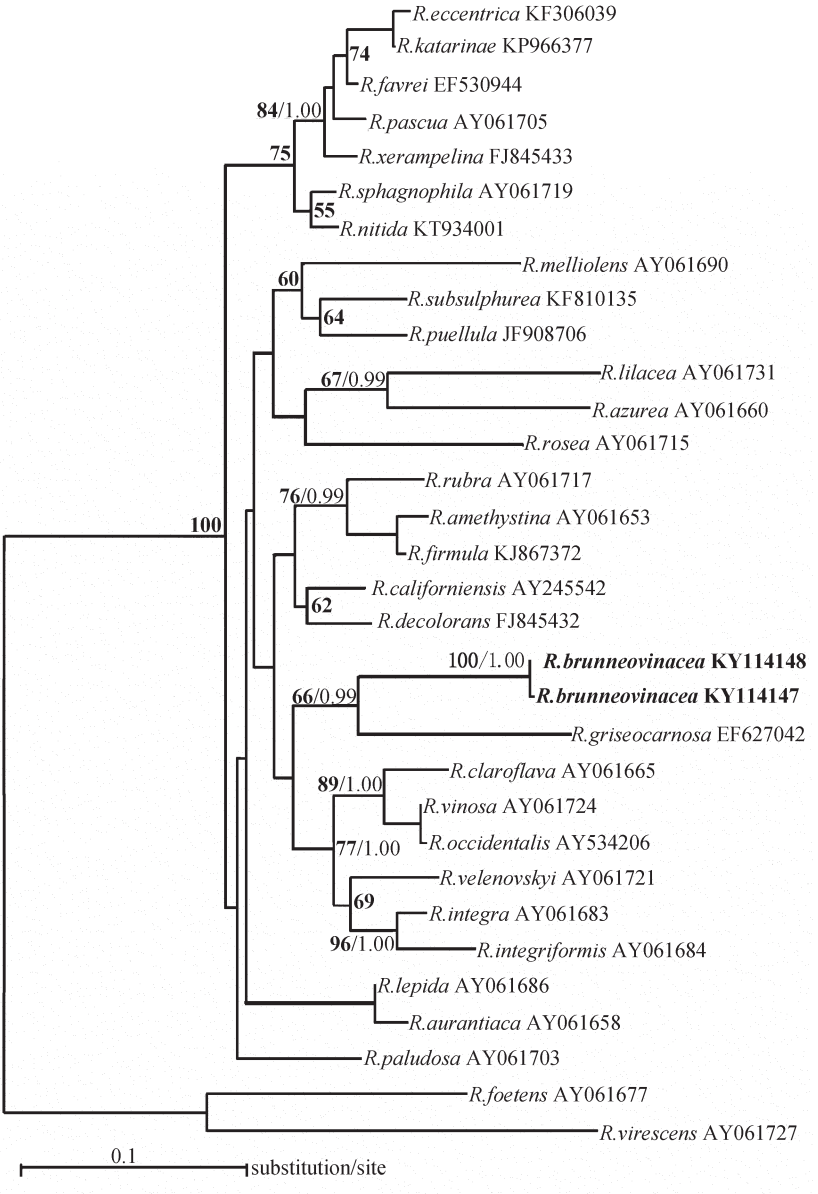

Fig. 2. The best RAxML likelihood tree (–ln L 3803.544073) based on the *Russula* ITS dataset. Support values in bold type are RAxML likelihood bootstrap (≥50%). Values in normal type are Bayesian posterior probabilities (≥0.95).

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